The University of Chicago

TESTICULAR HETEROTRANSPLANTATION IN RATS AND MICE

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1935

By

LUDVIG GUSTAV BROWMAN

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 75, No. 2, February 1937

The University of Chicago

TESTICULAR HETEROTRANSPLANTATION IN RATS AND MICE

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1935

By

LUDVIG GUSTAV BROWMAN

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 75, No. 2, February 1937

R. UNIV.
BIBLIOTHEEK
LEIDEN

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 75, No. 2, February, 1937

TESTICULAR HETEROTRANSPLANTATION IN RATS AND MICE [1]

LUDVIG GUSTAV BROWMAN
Hull Zoölogical Laboratory, The University of Chicago

TWO PLATES (EIGHT FIGURES)

INTRODUCTION

Various claims have been made that heterotransplanted testicles persist and are physiologically active in the host mammal for long periods of time. These claims have been vigorously denied by other investigators. Therefore further work upon the problem seemed desirable, and in this paper are presented the results of a series of testicle auto-, homo-, and heterotransplants in rats and mice.

The general operative procedures used in this work were similar to those reported by Moore ('26) and Crisler ('29). Whole testicles or portions of testicles were transferred to subcutaneous, peritoneal, scrotal, or intra-muscular sites from donors of various ages to hosts of various ages. Tissues were examined histologically following fixation in Bouin's fluid, paraffin embedding, and staining with Ehrlich's haematoxylin and eosin. The seminal vesicles were cut at 4 to 6 μ; testicles and grafts at 10 μ.

Rats and mice were used as experimental animals because the male hormone indicators in both have been developed to a highly dependable degree. The histocytology of the seminal vesicles gives an excellent means for detecting the presence of male hormone as found by Fisher ('23) and confirmed by others.

[1] This investigation has been aided in part by a grant from the Rockefeller Foundation to The University of Chicago.

A testicle transplant was considered to be a successful graft if it had persisted in the castrate host for a period of 15 or more days and appeared approximately histologically normal and healthy, and if, in addition, the male hormone indicators were positive. Lack of incorporation of a transplant was usually characterized by necrosis, fibrosis, resorption, calcification, or combinations of these conditions.

Host resistance to foreign bodies and foreign tissue transplantation has been said to be reduced as a result of organ extirpation or of the lowering of the activity of the reticulo-endothelial system by various procedures. Attempts were made in the present work to reduce host resistance to hetero-transplants by means of x-rays, splenectomy, adrenalectomy, and reticulo-endothelial blockade.

This problem was suggested by Dr. Carl R. Moore, whose advice, guidance, and helpful assistance are gratefully acknowledged. Acknowledgment is also made to Dr. Charles A. Shull of the department of botany for his kindness in making available the use of the x-ray apparatus. Dr. William Bloom of the department of anatomy was very helpful in interpreting certain slides, for which I am deeply appreciative. Dr. Audra A. Browman of the department of physiological chemistry kindly made chemical analyses of several transplanted tissues.

TESTICULAR AUTOTRANSPLANTATION

A number of auto- and homotransplantations were carried out on both albino rats and albino mice in order 1) to perfect a technique of transplantation; 2) to gain information regarding the reactions of testis transplants; 3) to observe the response of the host seminal vesicle to the various transplants; and 4) to determine preferential sites, etc., for testis transplantation.

A. Mice

Eight transplants in this category were made, of which four transplants were sutured to the animals' own inverted scrotal sacs and four were inserted into subcutaneous pockets. On autopsy all the grafts were found to be carrying on active spermatogenesis (fig. 1). Definitive spermatozoa were reached in all four of the scrotal grafts and primary spermatocytes were present in subcutaneous grafts. This is in agreement with the evidence presented by Moore and Quick ('24) indicating the temperature-regulating importance of the scrotum for complete spermatogenesis in certain mammals.

Examination of the seminal vesicles of the castrate mice with autografts revealed high secretory epithelium with abundant secretion granules similar to the seminal vesicles of normal males. The seminal vesicles of castrate control animals of the same age had low epithelium, absence of secretion granules, and no significant secretion in the lumen.

B. Rats

Six autotransplantations were made in this group, four of them to scrotal positions and two to subcutaneous sites. The grafts showed pictures similar to those secured from mice, with the exception that there were no definitive spermatozoa in the scrotal grafts. The seminal vesicles of the castrate rats with autografts resembled those of normal males in their secretory activity.

TESTICULAR HOMOTRANSPLANTATION

Homotransplantations of testicles were made into males, females, castrated males, and spayed females, upon animals ranging from 1 day to 6 months in age. Castrated males were used more than any other group since the seminal vesicles of the castrate hosts were utilized to indicate secretory activity of the graft. All experimental animals were random selections from the colonies of this laboratory.

A. Mouse testis transplantation into mice

Eight cases of homotransplantation of testicles in mice yielded seven well-incorporated secretory grafts (fig. 2). The seminal vesicles in all seven cases indicated practically normal secretory activity (fig. 3).

B. Rat testis transplantation into rats

Eighteen of the thirty-eight testis homotransplantations made in rats yielded well-incorporated grafts. Of the remainder, two grafts showed well-preserved tissues, but gave no evidence of hormone secretion; and the rest were either completely calcified, partially calcified and fibrotic, or entirely fibrotic. The well-incorporated grafts were recovered from thirteen castrate males, two normal males, and three normal females. The grafts of the last five animals mentioned were classified with the actively secreting grafts because of their general histological condition, although their hormone secretion could not be judged. The unsuccessful grafts were found in fourteen castrate males, three normal males, one spayed female, and two normal females. Hence the sex of the host apparently had little to do with the success of the transplant. Moore ('26), using a larger number of animals, found that spayed females gave a slightly greater percentage (95%) of persistent grafts than did castrate males (79%).

Thirteen grafts in castrated males produced seminal vesicles that indicated secretory activity approximating that of normal animals. The two animals with non-secretory grafts showed tissue containing viable epididymides, in addition to a large number of seminiferous tubules with Sertoli cells, and a few tubules with mitotic spermatogonia. The interstitial tissue present in these two cases appeared essentially normal, but the seminal vesicles were those of castrate animals. One of these animals had a transplanted seminal vesicle from a 1-day-old rat which also showed castrate type epithelium. Fisher ('23) reported that transplanted seminal vesicles in normal male rats presented normal appearing epithelium with secretion granules.

A considerable number of the recovered transplants were of a white chalky consistency and crumbled readily when crushed between the fingers. Rat 228, for example, which had been bilaterally castrated at 14 days of age and had had one-half of a testicle from a 65-day-old rat attached to the peritoneum, gave a characteristic white nodule at autopsy 46 days later. Upon sectioning, complete calcification was found. Several homotransplants gave such calcification reactions, although it was more commonly present in heterotransplants (fig. 8). Specimens of normal testicles and of calcified transplants were then analyzed for their inorganic salts. Ten normal mouse testicles contained traces of calcium and phosphorus (less than 0.1%), while four mouse testicle transplants removed from rats 63 days after transplantation contained an average of 9% calcium and 5% phosphorus. The calcium, carbonate, and phosphate in the transplants were present in about the same proportions in which they exist in bone. Histological examination, however, showed no signs of cartilage or of bone tissue. The analyses of rat testicles and grafts revealed a similar situation.

TESTICULAR HETEROTRANSPLANTATION

A. Rat testis transplantation into mice

Forty-five transplantations of rat testicles into mice hosts were made. Eleven failed to yield a graft; ten showed calcification; three exhibited considerable calcification and connective tissue replacement of dead tubules; fifteen gave small transplants that were essentially host connective tissue; and six yielded well-stained donor tissue. The seminal vesicles of the castrate hosts resembled those of bilaterally castrate animals, thus indicating that no detectable amount of hormones from the feebly viable transplants or from tissues undergoing autolysis was present. No transplant in this series was removed from the host before 30 days after the operation. Calcification was present in all the transplants recovered 5 to 5½ months after transference into the host.

A description of a few characteristic transplants may suffice to give an indication of the types of reaction of the transplant and host. Mouse 67 was castrated at 30 days of age, and had the lacerated testicle of a 3-day-old rat fastened to the right scrotal sac. Autopsy 5½ months later showed that the center of the transplant was entirely calcified. The tubules at the periphery of the calcified area had dead, pycnotic, and fragmented nuclei. There was a thick capsule about the entire graft, and in this capsule were scattered clusters of foreign body giant cells. Epithelioid Sertoli-like cells were present in peripheral invaded seminiferous tubules, and a few of these cells were present near the central necrotic pocket. The seminal vesicle was that of a castrate animal.

Mouse 42 was castrated at the age of 59 days and had one-third of the testicle of a 5½-month-old rat fastened to the right scrotal sac; autopsy was performed 67 days later. Although the transplant revealed dead and calcified tubules embedded in host connective tissue, the epididymal portion of the graft had normal-appearing epithelial cells, the lumen was patent, and the columnar epithelium appeared to be quite normal, both morphologically and in its staining reaction. Mummified sperm (fig. 7) were present in a few of the dead seminiferous tubules. (The term 'mummified sperm' is used to designate the persistent deep blue staining reactions of sperm heads or spermatozoa in otherwise necrotic or calcified tubules.) Lymphocytic activity was confined to a small pocket on one side of the transplant, and connective tissue had made but slight headway into a few tubules. Clusters of foreign body giant cells were present where the connective tissue had invaded and replaced the seminiferous tubules. Epithelioid Sertoli-like cells were present in the lumen of several partly calcified tubules. The seminal vesicle was of the castrate type.

Mummified or non-autolyzed spermatozoa were present abundantly in the peritoneal transplant of mouse 47. The tubules in this graft were all dead and a few peripheral tubules were calcified. In a few of the partly calcified tubules

epithelioid Sertoli-like cells were quite abundant. A graft giving quite a different picture from this one was the subcutaneous graft in a 29-day-old mouse of a testicle from a 24-day-old rat, autopsied 2 months after the transplantation. Practically all these tubules had been replaced with connective tissue, with the usual exception of a few peripheral calcified tubules.

In general it may be said that rat testes transplanted into mice hosts often showed a mass or nodule of tissue when examined 30 days to 5 months after transplantation. However, careful analysis failed to reveal a single graft out of forty-five transfers that showed well-incorporated normal-staining seminiferous tubules. In several cases the transplanted epididymides were retained in such a state that cells of their tubules were well stained. These were perhaps viable cells. In a small number of grafts certain epithelioid Sertoli-like cells possibly represented persistent donor Sertoli cells. Interstitial cells as such were not identified.

For the most part the grafts recovered after varied periods of time represented calcified and necrotic tissue of the transplant. The stained outlines of spermatozoa in the so-called mummified state could be seen in grafts removed as late as 5 or more months after transplantation. Hormone secretion as judged by the histological state of the seminal vesicle was not in progress in any graft in this series. The conclusion seems apparent, therefore, that the successful functional incorporation of rat testicles into mouse hosts is highly improbable.

B. Mouse testis transplantation into rats

One hundred and one cases of heterotransplantation of mouse testicles into rat hosts were made. Twelve of this number failed to yield a graft; thirty grafts were calcified, of which three completely calcified grafts were sectioned to verify macroscopic and chemical diagnosis; fourteen grafts showed partial calcification and fibrosis; five showed relatively complete replacement of dead tubules by connective

tissue without calcification; and forty grafts were considered as non-functional incorporated grafts. Considerable amounts of donor tissue retained a certain amount of differentiation and was almost normal in its staining reaction.

As mentioned previously the sex of the host apparently had little to do with the persistence of a graft. The greatest number of persisting grafts were recovered from the younger hosts, with a progressive decrease in recovered tissues from the older animals. The positions of the transplants were without significance since the proportion of persisting grafts in the various sites was roughly proportional to the number of transplants in those sites. All the transplants had been in the host for at least 30 days, most of them had existed in the foreign host for at least 40 to 50 days, a few up to 70 days, and two over 5 months. Tissues present in the host 30 to 70 days showed well-stained donor tissue upon recovery. The two cases of transplants residing in the host for over 5½ months consisted of masses of dead and calcified tubules, with stainable 'mummified' spermatozoa in many of the peripheral tubules. The seminal vesicles failed to give any indication of hormone secretion from any graft in this series.

A few representative grafts will be described to give the general picture of the persisting types of grafts. Rat 113 was made unilaterally castrate at 30 days of age, and had a lacerated testicle from a 29-day-old mouse sutured to the right scrotal sac. Autopsy 41 days later showed the graft to have a thin host capsule and a necrotic donor capsule, internal to which was a scattered periphery of calcified tubules. A few peripheral tubules were invaded by connective tissue. Just within the calcified layer was a narrow zone of dead tubules in some areas, while in others these tubules showed faintly staining nuclei (fig. 5). The larger portion of the central area of the graft contained practically normally staining nuclei of interstitial cells and seminiferous tubular elements (fig. 6). The lumina of the tubules were filled with cells. Spermatogonia and spermatocytes were present, but no spermatids or spermatozoa. It appeared almost as if

development had been stopped at 29 or 30 days of age or shortly after the time of transplantation. No blood or lymph vessels could be seen leading into the central area of tubules, or into the transplant proper.

Rat 155 was made bilaterally castrate at 10 days of age and had a testicle of a 34-day-old mouse fastened to the peritoneal wall. At autopsy 62 days later the graft was found to be enclosed in a thin capsule internal to which was a zone of calcified tubules. Embedded in one-half of the graft were scattered calcified tubules, aggregations of foreign body giant cells and epithelioid Sertoli-like cells (fig. 8). The other half of the graft was essentially a mass of dead, necrotic, and partly calcified tubules with mummified sperm present in the lumen of the dead tubules. The sperm seemed equally well preserved in the inner tubules as in the peripheral tubules although fewer tubules in the inner portion of the transplant contained spermatozoa. A sharp line of demarcation was present between the necrotic half and the invaded half of the graft. This line seemed to be characterized by foreign body giant cells intermixed with epithelioid Sertoli-like cells.

Rat 199 was made bilaterally castrate at 38 days of age and received the macerated testicles of a 50-day-old mouse in a subcutaneous pocket. Autopsy 68 days later showed a thin capsule, a periphery of calcified tubules, partial invasion by connective tissue, and well-stained tubules in the major portion of the grafts (fig. 4). Clusters of epithelioid Sertoli-like cells and foreign body giant cells were scattered throughout the grafts, especially in the partly calcified tubules embedded in connective tissue. Sperm heads were present in peripheral dead tubules. The seminal vesicle was that of a castrate animal.

In general, in the heterotransplantation of mouse testicles into rats, nodules or masses of tissue were recovered at autopsy as long as 6 months after transplantation. Blood vessels were usually present in the capsule of the nodule, but blood and lymph vessels seemed to have great difficulty in reaching the transplant proper and were never found in donor

or graft tissue. Calcification of whole or portions of the transplant was so common that most of the heterotransplanted tissues recovered were included in this category. The most characteristic reaction of the better preserved grafts was the zonation reaction (figs. 4 and 5), or 1) an outer host capsule, 2) necrotic donor capsule, 3) peripheral zone of calcified or partly calcified seminiferous tubules, 4) zone of faintly staining tissue continuing into 5) the central mass of comparatively well-staining tubules. Spermatozoa in the form of sperm heads were present in dead tubules as late as 5½ months after transplantation in otherwise homogeneously stained and necrotic transplants. The epididymides of a number of grafts remained in a condition that permitted the cells to be well stained for as long as 2 months after transplantation. The cells were possibly viable.

In spite of the appearance of well-stained tubules and interstitial cells in a large number of grafts, hormone secretion was not in progress in any transplant of the heterotransplanted series as indicated by the histological condition of the seminal vesicles. The conclusion seems justified that successful or functional incorporation of heterotransplanted testicles between rats and mice is highly improbable.

TRANSPLANTATION TO TREATED ANIMALS

A series of animals were subjected to various experimental treatments at about the same time that they received testicle transplants, in an attempt to modify the host resistance.

A. Treated mice

1. Mouse testes held in physiological saline. Stainable spermatozoa and feebly staining nuclei of other testicular elements had been found in grafts which had resided in foreign hosts for long periods of time. It was therefore thought advisable to study normal testicles removed from the body and placed in physiological saline at different temperatures for varying lengths of time to see to what extent the tissue retained its approximately normal staining qualities.

Composition of saline solution

	gm.		gm.
NaCl	8.000	$MgCl_2 \times 6\ H_2O$	0.239
KCl	0.084	KH_2PO_4	0.052
$NaHCO_3$	0.040	$Na_2HPO_4 \times 2\ H_2O$	0.143
$CaCl_2$ (anhyd.)	0.048	Dextrose, C.P.	1.000

in 1 liter of distilled water; sterile.

A series of normal adult mouse testicles were placed in sterile saline solution, and the saline was replaced daily with a fresh supply. One set of testicles in saline was placed in an electric refrigerator at a temperature of about 4°C., a second was kept at room temperature at approximately 20°C., and a third was placed in an incubator at about 37° to 38°C. The tissues were fixed in Bouin's fluid on the second, seventh, eleventh and twentieth days.

As would be expected, the testicles kept in the incubator showed the most severe changes, for at the end of the second day shrinkage of all tubules was apparent, almost all the nuclei were pycnotic and granulated, and cytoplasmic differentiation was lost. All that remained was essentially a homogeneously staining mass about a syncytium of pycnotic nuclei in the tubules. All the germinal cells were stained although many nuclei only feebly so. There was abundant liquefaction as indicated by the large vacuolated areas. By the end of the seventh day the only stainable nuclei were those of spermatids and spermatozoa, and a few very feebly stained spermatocytes and interstitial cells. This was also essentially true on the eleventh day. Tubules by this time were vacuolated to a much greater extent.

The testicles kept at room temperature passed through the same picture as described above only at a much slower rate. After 7 days the testicle was comparable to a 2-day testicle of the incubated set. By 11 days there was still an abundance of well-stained spermatids and spermatozoa with a few faintly staining spermatocytes.

The testicles placed in the ice box retained essentially a normal picture with numerous mitotic figures still present at the end of 2 days. At 7 days most of the nuclei were pycnotic; cells in all stages of spermatogenesis stained well as

did the interstitial cell mass. Essentially the same picture was seen at 11 days with all stages of spermatogenesis and interstitial cells stained. Vacuoles appeared in considerable numbers at this time. By the twentieth day considerable shrinkage was apparent in all tubules, and they were essentially homogeneous in their staining reaction. Spermatids and spermatozoa stained very well; occasional pycnotic Sertoli cells, spermatogonia, and interstitial cells were almost normal in their appearance and staining reactions. Spermatozoan heads stained the strongest of all the cells studied.

The fact that even occasional Sertoli cells, spermatogonia, and interstitial cells were almost normal in their staining reactions 20 days after the whole testis had been held in a nutrient alkaline solution outside the body, causes one to be a bit more hesitant about ascribing successful incorporation to transplanted non-vascularized tissues which had been in an environment containing fluids of a similar nature. The persistence of spermatozoa in a grafted testicle cannot be used as a criterion for a successful functional graft.

Following the same general plan as outlined above, a few testicles that had been placed in the refrigerator, incubator, or room temperature for varying periods of time were autotransplanted. Only eight cases were tried. Two of the animals died shortly after the operation, and in three animals no tissue was recovered. Of the three animals from which tissue was recovered only one animal had well-incorporated grafts. These transplants were from two testicles that had been placed in the refrigerator and at room temperature respectively on one day, and had been macerated and autotransplanted subcutaneously on the following day. The transplants had remained in the host 41 days before autopsy. Both grafts contained Sertoli cells, spermatogonia, and spermatocytes, although most of them were in a pycnotic condition. Both of these grafts contained giant cells, leucocytes, and lymphocytes in scattered pockets; the whole grafts were well encapsulated and invaded with connective tissue. The seminal vesicles had low epithelium with only a small

amount of secretion granules. These few transplants failed to indicate more than that mouse testicles can be successfully autotransplanted after 24 hours in a refrigerator or at room temperature.

2. Mouse testes held in rats before autotransplantation. Loeb ('35) called attention to the action of the so-called heterotoxins of one species against tissue transplants of another species and of the inhibiting and lethal action of such toxins on the transplant. It is possible that foreign tissues transplanted to another species may lie dormant for a long time without significant autolysis occurring before any antagonistic host reactions are developed. Whether a protein of one species reacts quickly against the proteins of a foreign tissue or whether such antagonistic reaction must be built up gradually within the host would be indicated by the transfer of a given tissue from one species to another species. The viability of the transplanted tissue could be determined by retransplantation to the original donor. Such a method might give the threshold of reaction time of any heterotoxins developed or the limits of life of a tissue away from its normal environment.

Seven male mice, 35 days old, had their testicles removed and immediately transplanted subcutaneously to rats of 33 days of age. From 1 to 5 days thereafter testicles were removed from the rat hosts and retransplanted into subcutaneous pockets of the original male mouse donor. All transplants were recovered on autopsy about 40 days later. Only three grafts were sectioned, those from 1, 3 and 4 days' delayed transplantation. Each graft was well-incorporated, with viable cells ranging from tubules with a syncytium of Sertoli cells with scattered spermatogonia to tubules with almost normal spermatogenesis up to and including primary spermatocytes. The seminal vesicles in all three cases had medium to high secretory epithelium with secretion granules. Placing testicles from one species (mouse) into another species (rat) for as long as 4 days did not prevent the testicles from becoming well-incorporated functional grafts when returned to the original donor.

It would therefore seem reasonable to assume that the unsuccessful incorporation of heterotransplanted testicles was not due to the inability of the testicle to survive the relatively deleterious influences of 1) immediate lack of vascularity, 2) unfavorable temperature, or 3) the immediate exposure to foreign tissue fluids.

3. Rat testes transplanted to treated mice. Rat tissue extract. A series of mice were treated with saline extracts of macerated organs of young rats (1 to 3 days old) for several days after rat testicle transplantation. Liver, kidneys, adrenals, urinogenital tract, spleen, and muscle were ground in physiological saline and squeezed through several thicknesses of cheesecloth. This freshly prepared solution or suspension was injected daily into the host. Grafts recovered after 40 days revealed almost uniform calcification and fibrosis. The best graft was one in which the peripheral tubules were filled with pycnotic nuclei and cell debris embedded in a mass of fibrous connective tissue and lymphocytes which surrounded a central core of dead and calcified tubules. Connective tissue invasion and lymphocytic and leucocytic invasion were well advanced in all cases. The indications were that host resistance had been increased rather than decreased by the treatment.

Reticulo-endothelial blockade. An attempt was next made to load the reticulo-endothelial system of the host with inert foreign particles for at least 10 days following the transplantation of testicles. A 2% solution of Higgins' India ink in a physiological saline solution was injected in $\frac{1}{2}$ cc. doses daily, either intraperitoneally or intramuscularly. The recovered grafts indicated no significant difference between the reactions of India ink-injected and non-injected hosts to grafts. The grafts were well invaded by host connective tissue, with some lymphocytic and leucocytic activity still persisting 40 days after the transplantation was made. This treatment apparently did not delay host resistance.

Adrenalectomy. Six mice were bilaterally adrenalectomized, castrated, and given rat testicle transplants. The one

animal living to the autopsy date had part of the left adrenal regenerated. The recovered grafts had well-stained cells in the epididymides, stained pycnotic germinal cell elements, and pycnotic nuclei scattered throughout the central degenerating portions of the grafts. The usual zonation reaction in heterografts was not present, and there were practically no tubules with basement membranes or connective tissue outlines. Leucocytes had invaded the grafts and were widely dispersed. The seminal vesicle was that of a typical castrate.

Seven mice were unilaterally adrenalectomized, castrated, and given rat testis transplants. India ink suspension was injected subcutaneously for 6 days following the operation. The five grafts recovered failed to show any well-stained donor tissue. The grafts were well invaded by host connective tissue, although there were pockets of dead donor tubules that had not been replaced by host connective tissue. Calcification and fibrosis were present to about the same extent in these grafts as they had been in grafts from mice hosts not so treated.

Splenectomy. Ten mice were splenectomized, castrated, and given rat testicle transplants. They were injected intramuscularly with ¼ cc. of a 2% India ink suspension for 10 successive days following the operation. Seven grafts were recovered at autopsy about 42 days later. Calcification, necrosis, and connective tissue invasion were not different from that in non-splenectomized or non-treated animals. No well-stained donor tissue was recovered.

Splenectomy and unilateral adrenalectomy were performed at the same time that the host mouse was castrated and given transplants of rat testicle substance. Only eight of fourteen animals operated upon yielded grafts. Five of these grafts showed partial or complete calcification; two were completely replaced by host connective tissue; and one contained persistent dead tubules encapsulated in a heavy layer of connective tissue with a scattering of epithelioid Sertoli-like cells in peripheral tubules.

X-ray treatment. An attempt was next made to reduce the lymphoid tissue and cells of the reticulo-endothelial system in the host by means of Roentgen rays. Ten mice (30 to 38 days of age) were exposed to x-rays while held in a cardboard box about 21 cm. × 17 cm. × 10 cm. high. The animals were permitted complete freedom within this container, and were exposed 5 minutes daily. The Roentgen units per 5 minutes exposure were approximately 32.34 R. A 3-mm. aluminum filter was used, the target object distance was 40 cm. measured from the bottom of the box, the tube current was 5 milliamperes, and the tube voltage was about 100 kv. peak. On the fourteenth and last day of exposure no filter was used, the target object distance and the tube current remained the same, but the tube voltage was reduced to 60 kv. peak, thus giving a dosage of about 150.53 R. units for the 5-minute exposure. The total dosage for the entire period of exposure was about 570.93 R.

The x-ray dosage, castration and transplantation were apparently too severe on the animals. None of them survived more than a week following the operation (ether anesthesia, tincture of iodine and 70% alcohol for asepsis, drafty animal room); the control non-operated x-rayed mice were alive 5 months after the exposure.

B. Treated rats

All the auto- and heterotransplants made to rats with extirpated organs other than gonads and those receiving x-ray treatment are included within this series. All animals were castrate males with the exception of one normal female. All transplants were placed in subcutaneous pockets. The age of the hosts ranged from 7 to 21 days at the time of transplantation in the non-x-rayed series and 28 to 42 days in the x-rayed series. The age of the donors in the non-x-rayed groups ranged from 7 to 21 days, and in the x-rayed group from 30 to 64 days. All transplants in the non-x-rayed group remained in the host 37 to 45 days after transplantation, and in the x-rayed group were removed at intervals of 5 days until the terminus of 45 days was reached.

1. Rat testes held in mice before autotransplantation. The effect on the survival of rat testicles isolated for varying periods of time in a foreign species before autotransplantation was also tested in the rat. The seven transplants resulted in two resorptions, two grafts represented by masses of connective tissue, and three well-incorporated functional grafts. These latter grafts had remained 2, 4 and 4 days, respectively, in subcutaneous pockets of mice before transplantation. All three grafts had tubules containing Sertoli cells and spermatogonia, and two of them had tubules with spermatocytes. These grafts were from 5- to 7-day-old rats transplanted to 18- and 20-day-old mice 2 to 4 days before autotransplantation. The grafts were retained in the final host a minimum of 37 days. The seminal vesicles showed high epithelium with secretion granules.

2. Mouse testes transplanted to treated rats Mouse tissue extract. The effect of mouse tissue extract upon heterospecies testicle incorporation was tried on rats. The rats were injected with mouse extract (prepared as previously described) every other day for a period of six injections following transplantation of testicles from 2-week-old mice. The grafts were permitted to remain in the host for about 45 days. All the grafts were almost entirely calcified although they contained a few epithelioid Sertoli-like cells in the peripheral partly calcified tubules. All the seminal vesicles were of the castrate type.

Reticulo-endothelial blockade. Injections of India ink suspension in saline were tried on several rats receiving heterotransplants. The recovered grafts revealed no stained donor tissue, but showed calcified and necrotic tubules with slight connective tissue invasion.

Adrenalectomized rats with transplants of mouse testicles died within 10 days of the operation. Not a single graft was recovered from bilateral adrenalectomized hosts. Five animals were unilaterally adrenalectomized, castrated, and given transplants of mouse testicles in subcutaneous pockets. The hosts were also injected intramuscularly with ¼ cc. of a 2%

India ink suspension for 6 days. The grafts recovered were represented almost entirely by host connective tissue.

Splenectomy and ten daily injections of an 8% India ink suspension in saline were carried out on eleven castrate rats receiving heterotransplants. Six grafts were recovered and all of them showed partial or complete calcification, scattered calcified tubules embedded in connective tissue, persisting pycnotic interstitial cells, and foreign body giant cells and Sertoli-like cells in peripheral dead and calcified tubules.

Splenectomy and unilateral adrenalectomy were carried out on eleven castrated male rats receiving mouse testicle transplants. Seven grafts were recovered at autopsy about 43 days later. Peripheral calcification of tubules with central masses of dead tubules gave the characteristic picture. No well-stained donor tissue was found.

X-ray treatment. Ten rats were exposed to x-rays for 14 days as described above, and were then castrated and given mouse testicle transplants on the second day following the last exposure. One of the rats died within a week after the operation. All the other rats survived the duration of the experiment, as did the control non-operated x-rayed rats. Normal non-x-rayed mouse testicles (63 to 64 days of age) and testicles from x-rayed mice (30 to 31 days of age) were transplanted subcutaneously into x-rayed rats. Each animal received four transplants, i.e., two normal and two x-rayed. Five days after transplantation the x-rayed testicle graft showed spermatogonia in mitosis. At 11 days pycnotic interstitial cells and Sertoli cells were still in evidence and from the fifteenth day to the fortieth only epithelioid Sertoli-like cells embedded in host connective tissue and calcified tubules were present. The transplants from the older non-x-rayed mice donors produced grafts with pycnotic interstitial cells and spermatogonia up to about the fifteenth day. Epithelioid Sertoli-like cells were present in all grafts up to about the forty-fifth day. No definitive spermatogonia, germ cells, or interstitial cells that could be recognized as donor tissue were present after the fortieth day. The seminal vesicles of all animals x-rayed were of the castrate type.

The grafts recovered from treated animals indicated that the host reaction against the transplant had not been significantly altered in either direction. Indications were that the forces antagonistic to heterospecies grafts had a threshold of activity far beyond that which could be affected by the methods employed.

TABLE 1

Summary of all testis transplants

CATEGORY	HOST	NUMBER OF CASES	RECOVERED	PERCENTAGE RECOVERED	HORMONALLY ACTIVE	HORMONALLY INACTIVE	FIBROTIC NECROTIC CALCIFIED RESORBED	DURATION OF TRANSPLANT IN DAYS
Auto-	Rat	6	6	100	5	1	..	41– 42
transplant	Mouse	8	8	100	8	..	..	41– 52
Homo-	Rat	38	34	89	18	2	18	20– 48
transplant	Mouse	8	8	100	7	..	1	38– 62
Hetero-	Rat	101	89	88	..	40	61	35–168
transplant	Mouse	45	34	77	..	6	39	30–167
Treated hosts								
A. Auto-	Rat	9	7	77	3	1	5	37– 41
transplant	Mouse	15	11	73	5	..	10	37– 41
B. Hetero-	Rat	57	33	57	..	6	51	6– 45
transplant	Mouse	66	34	51	..	3	63	38– 46
Totals		353	264	81	46	59	248	.....

DISCUSSION

The present data show that the auto- and homotransplantation of testicles do cause the reactivation of castrate seminal vesicles almost to a normal state in both rats and mice. The heterotransplantation of testicles, on the other hand, does not cause any detectable response in seminal vesicles examined 15 or more days after transplantation. Moore ('30) using the rat seminal vesicle test and the sperm motility test for male hormone indicators states that no detectable hormone could be demonstrated in animals with large implanted testicles undergoing autolysis. Voss ('34), using the mouse seminal vesicle test, has demonstrated in the castrate mouse that repeated implantation of non-living macerated or whole testicular transplants undergoing autolysis are capable of

producing detectable quantities of male hormone for about 9 days after the first implants. The work of both of these men clearly indicates that insufficient hormone was being produced by testicles undergoing autolysis to be of any significant therapeutic value. If the results of these workers and the data presented in this paper can apply to higher mammals it seems quite reasonable to assume that any beneficial effects claimed for testicle heterotransplants in man are probably not the result of hormone liberation.

Geneticists have long recognized that compatibility and incompatibility of transplants are directly tied up with the nearness or similarity of the genetic constitution of host and transplant. Loeb and Wright ('27) demonstrated that brother to brother tissue transplants from inbred guinea pig stocks had about the same degree of success as tissues that were autotransplanted in the same stocks. Brother to brother transplants from non-inbred strains took less well than transplants of any category of the inbred family. This is accounted for on the basis of the comparative homogeneity of the inbred stock, and therefore the reaction against the transplanted tissue in the host depended upon the genetic factors of the donor. The number of strange genes of the transplant was believed to determine the severity of the host reactions against the transplant. It was apparently the presence of strange genes of the donors and not their absence which acted as stimuli to the host and incited the host reaction. This difference of genetic constitution is well represented in rats and mice, in which there has apparently been an extensive shifting of chromatin material and genetic constitution since the origin of the separate species. (The white rat has a diploid chromosome number of forty-two as compared with forty in the white mouse.) Transplantation between the two species is thus likely to encounter severe antagonistic reactions, and any possibility of successful transplantation would be highly doubtful.

The problems involved in the successful incorporation of a transplanted piece of tissue in a strange environment are

1) the genetic relationship of host and donor; 2) the presence or absence of hostile tissue fluids and accelerated cellular activity of the host, and 3) the problem of attachment and nourishment of the transplant. The general defense reactions of the host to foreign bodies, although basically chemical in nature, elicit responses of the entire reticulo-endothelial system, leucocytes, monocytes, and usually invasion by fibroblasts. Attempts have been made to modify this host resistance by various methods with variable results. Tinozzi ('31) and others claim that spleen homotransplantation increases the resistance of hosts to foreign transplants. Arloing, Morel, Josserand and Badinande ('33) claim that injections of adrenal cortical substance increase host resistance to foreign bodies. Splenectomy, bilateral adrenalectomy, and the injection of inert foreign particles into the host blood stream are said to reduce the resistance of the host to foreign bodies, probably by acting through the blockade of the reticulo-endothelial system. X-ray treatment given in proper dosages has been reported to permit transplantation of foreign tissues into animals normally immune to such transplants. Bischoff and Maxwell ('32) insist that splenectomy, adrenalectomy and thyroparathyroidectomy cause no decrease or increase in the susceptibility of the host to foreign bodies. There was no noticeable increase or decrease in the host reaction toward heterografts in animals which were adrenalectomized, splenectomized, or x-rayed as reported in the present investigation.

A considerable number of the heterografts recovered were calcified or had persistent non-vascularized well-stained tissue. Retterer and Alexandresco ('34) reported 'mummified tubules' and spermatozoa in a transplanted monkey testicle that had remained in a human patient for over 3 years. They also reported tubular outlines and stained connective tissue in chimpanzee testicle grafts that had persisted in human patients for $4\frac{1}{2}$ and 6 years, respectively. The entire stained central portions were surrounded by necrotic non-vascular tissue in these anthropoid grafts. Crisler ('29) reported

'anatomically persisting' tubules in mouse testicles transplanted to rat hosts. This calcification phenomenon and the persistent staining reactions of transplanted tissue still lack an explanation.

It is a well-known fact that deposits of calcium salts occur in those places in which the tissue metabolism is low, such as in necrotic cells and tissue, cells with greatly reduced activity, or areas that are anemic or non-vascularized such as the testicle grafts between different species. When necrosis occurs without proper absorption the area is favorable to calcium deposits, and with the slow seepage of lymph and the absence of rapid oxidative changes a gradual calcification occurs. An increased blood supply results in a decrease in calcification. If the blood supply is completely shut off unchanged calcification follows (Jones and Roberts, '34). Demonstrable depositions of calcium are said to be present 36 hours after a temporary anemia of only 1 hour in the kidney (Wells, Holmes and Henry, '11). Calcification is obtained in lung and kidney tissue of rats within 48 hours after being placed in a solution of the inorganic salts of bone (Rosenheim and Robison, '34).

From data presented in the experimental portion of this paper and data and interpretations found in the literature, a tentative explanation is suggested for the persisting heterografts. It is possible that the transplants in a non-vascular area are in an alkaline medium greater than that of the blood, as indicated by the periphery of calcified tubules in a large number of the grafts. Since it has been shown by a number of workers that in an alkaline medium tissue autolysis is inhibited or retarded (Dernby, '18), it is conceivable that there is such an inhibition of autolysis taking place in these grafts. Sufficient lymph and body fluids from the host probably seep through to maintain approximately viable tissue as indicated by the staining reactions. This slow seepage of lymph probably causes the continuation of calcium deposits. It is possible in some cases that the antagonistic forces between the two tissues are so great that the transplanted tissue

dies completely very early and is gradually replaced by host connective tissue. Where an entire transplant has been calcified, the calcification process has been more or less continuous with little or no host connective tissue invasion. Occasional grafts are found in which the tubules only are calcified and the intertubular areas completely invaded by host connective tissue. In these calcified grafts it is probable that more and more of the tubules deposit calcium salts and the calcified area remains relatively unchanged due to the complete absence of any blood supply. Spermatozoa which persist in a mummified state up to as long as 5 or more months in dead tubules may perhaps indicate the influence of cellular cytoplasm and contained autolytic enzymes.

SUMMARY AND CONCLUSIONS

1. Autotransplantation of testicles of rats and mice to the scrotal sac permits the development of definitive spermatozoa in the graft.

2. Scrotal, subcutaneous, and peritoneal auto- and homotransplants of testicles produce grafts that carry on spermatogenesis, and secrete hormone in sufficient amounts to maintain the seminal vesicles of a castrate host in an approximately normal state of activity.

3. The epididymides in heterografts persist in a fair state of preservation for as long as 2 months after transplantation. Spermatozoa, apparently mummified, may be found in dead tubules of heterografts recovered more than 5 months after transplantation.

4. Portions of, or entire testicle transplants may undergo calcification in auto-, homo-, or heterotransplanted testicles, although such calcification phenomena are more commonly found in heterotransplanted tissues.

5. Testicle heterografts which may show histologically well-stained cells and tissue are not demonstrably physiologically functional. Heterografts recovered as long as 2 months after transplantation and containing well-stained tissue may be without histological evidence of vascularization. The seminal

vesicle in no case of a bilateral castrate host with heterotransplanted testicles gave other than the castrate type of secretory epithelium.

6. Testicles placed in physiological saline and kept at 4°C., 20°C. and 37°C. continued to show well-stained spermatids and spermatozoa for 11 days. Testicles held for 20 days at 4°C. presented well-stained spermatozoa, spermatids, and occasional pycnotic spermatogonia, Sertoli and interstitial cells.

7. Testicles of one species held in the body of another species for as long as 4 days and returned to the original donor may become incorporated as functional grafts.

8. Splenectomy, adrenalectomy, tissue extracts, India ink injections, or x-ray treatments in these experiments did not markedly increase or decrease the resistance of host animals to heterotransplanted grafts.

9. The conclusion seems, therefore, justified that the heterotransplantation of testicular material between rats and mice does not yield successful or well-incorporated functional grafts.

10. If the results obtained from the transplantation of testicles between rodents can apply to higher mammals, it seems quite reasonable to assume that any beneficial effects claimed for such testicle heterotransplants are probably not due to the production or liberation of male hormone from such transplants.

LITERATURE CITED

ARLOING, F., A. MOREL, A. JOSSERAND AND A. BADINANDE 1933 Nouvelles recherches sur les propriétés antitumorales d'estraits de surrénales. Compt. rend. Soc. de Biol., T. 112, pp. 156–157.

BISCHOFF, F. AND L. C. MAXWELL 1932 Hormones in cancer. V. Effect of glandular extirpation upon the growth of transplantable tumors. J. Pharm. and Exp. Therap., vol. 46, pp. 51–58.

CRISLER, G. 1929 The heterogenous testis transplant problem as applied to white rats and mice. Am. J. Physiol., vol. 90, pp. 623–630.

DERNBY, K. G. 1918 A study of autolysis of animal tissues. J. Biol. Chem., vol. 35, pp. 179–219.

FISHER, N. F. 1923 Influence of the gonad hormones on the seminal vesicles. Am. J. Physiol., vol. 64, pp. 244–251.

JONES, R. W. AND R. E. ROBERTS 1934 Calcification, decalcification and ossification. Brit. J. Surg., vol. 21, pp. 461–499.

LOEB, L. 1935 Transplantation of tissues from mouse to rat and vice versa. Am. Nat., vol. 69, pp. 239–244.

LOEB, L. AND S. WRIGHT 1927 Transplantation and individuality differentials in inbred families of guinea pigs. Am. J. Path., vol. 3, pp. 251–283.

MOORE, C. R. 1926 On the properties of the gonads as controllers of somatic and psychical characteristics. IX. Testis graft reactions in different environments (rat). Am. J. Anat., vol. 37, pp. 351–416.

———— 1930 The physiologic effects of non-living testis grafts. J. Am. Med. Assn., vol. 94, pp. 1912–1915.

MOORE, C. R. AND W. J. QUICK 1924 The scrotum as a temperature regulator for the testes. Am. J. Physiol., vol. 68, pp. 70–79.

RETTERER, E. AND G. ALEXANDRESCO 1934 Étude descriptive des Greffons testiculaires. Rev. de Path. Comp., T. 34, pp. 821–847.

ROSENHEIM, A. H. AND R. ROBISON 1934 Calcification in vitro of lung, kidney, and aorta. Biochem. J., vol. 28, pp. 712–718.

TINOZZI, F. P. 1931 Über die Einwirkung der Milztransplantation auf das Verhalten tierischer Tumoren. Zeitschr. f. Krebsforsch., vol. 34, pp. 148–158.

VOSS, H. E. 1934 Über die Funktionsbedingungen von Hodentransplantaten. Arch. f. Entwick.-mech., vol. 131, pp. 82–94.

WELLS, H. G., H. F. HOLMES AND G. R. HENRY 1911 Studies on calcification and ossification. J. Med. Res., vol. 25, pp. 373–392.

PLATE 2

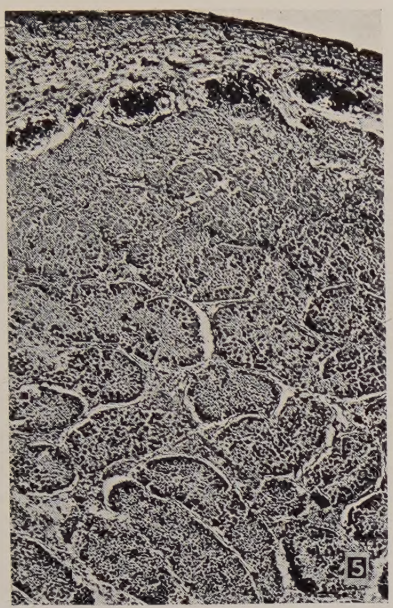
EXPLANATION OF FIGURES

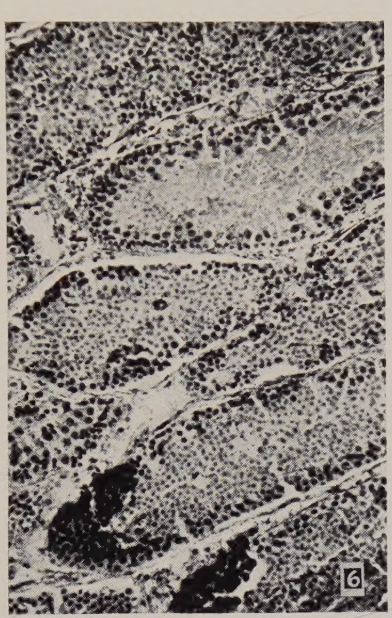
5 Rat 113; 41-day scrotal heterograft; zonation phenomena with stronger central staining reaction; dead periphery. × 130.

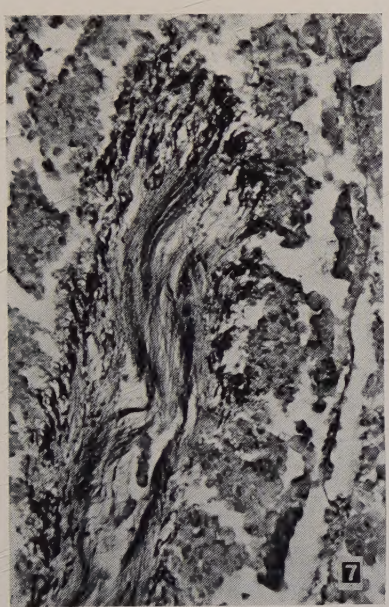
6 Rat 113; 41-day scrotal heterograft; higher magnification of above section; pycnotic nuclei. × 325.

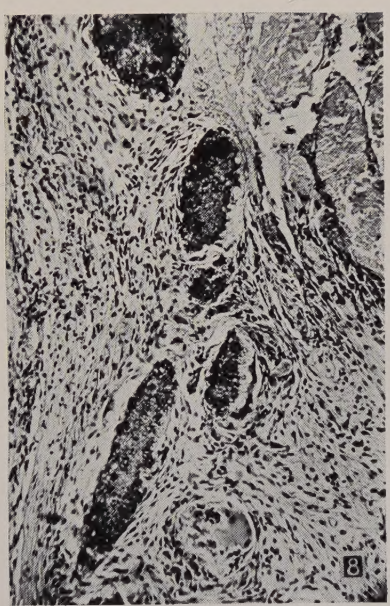
7 Mouse 47; 82-day peritoneal heterograft; mummified spermatozoa in necrotic tubules. × 525.

8 Rat 155; 61-day peritoneal heterograft; periphery of calcified tubules; part of graft invaded directly by connective tissue; other half of dead tubules in process of invasion. × 210.


5


6


7


8